SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

The connection between ventricular fibrillation and autophagy

by Andras Czegledi

Supervisor:
Dr. Istvan Lekli, PharmD. PhD

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF PHARMACEUTICAL SCIENCES

DEBRECEN, 2019

The connection between ventricular fibrillation and autophagy

By Andras Czegledi(MSc degree)

Supervisor: Dr. Istvan Lekli, PhD

Doctoral School of Pharmaceutical Sciences., University of Debrecen

Head of the **Examination Committee**:	Dr. Lajos Gergely, DSc
Members of the Examination Committee:	Dr. Béla E. Tóth , PhD
	Dr . AnikóPósa , PhD

The Examination takes place at Department of Pharmacology, Faculty of Pharmacy, University of Debrecen, 9 a.m., October 9, 2019

Head of the **Defense Committee**:	Dr. Lajos Gergely, DSc
Reviewers:	Dr. Csaba Csonka, PhD
	Dr. Sándor Somodi, PhD
Members of the Defense Committee:	Dr. Lajos Gergely, DSc
	Dr. Csaba Csonka, PhD
	Dr. Anikó Pósa, PhD
	Dr. Sándor Somodi, PhD
	Dr. Béla E. Tóth, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 1 p.m. October 9, 2019.

Table of Contents

I. Introduction and aims 4
II. Materials and methods 6
III. Results 10
IV. Conclusion 12

I. Introduction and aims

Nowadays the cardiovascular diseases (CVDs) are the number one cause of death globally, more people die annually from CVDs than from any other cause. Cardiovascular diseases are disorders of the heart and blood vessels and include coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions. Four out of five CVD deaths are due to heart attacks and strokes. Individuals at risk of CVD may demonstrate raised blood pressure, glucose, and lipids as well as overweight and obesity. Most cardiovascular diseases can be prevented by addressing behavioural risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol using population-wide strategies. In the European Union the CVDs cause 1.8 million deaths each year which account 37% of all deaths in the EU. Death rates from both ischaemic heart disease (IHD) and stroke are generally higher in Central and Eastern Europe than in Northern, Southern and Western Europe. According to the latest WHO data published in 2017, coronary heart disease deaths in Hungary reached 38,113 or 34.09% of total deaths. The age adjusted death rate is 181.46 per 100,000 of population ranks Hungary 31st in the world. The myocardial infarction or heart attack is the worst outcome of CVDs. During heart attack the blood flow decrease or stops to a part of the heart (ischaemia), causing damage to the heart muscle. The energy and oxygen supply in the heart muscle stop and begins anaerob metabolism then, after the blood circulation is restored (reperfusion) the oxidative stress causes different problems. The formation of reactive oxygen species can damage the cell's proteins, and organs; the mitochondrial transport system released and became harmful to the intracellular milieu. These deleterious phenomenons could lead to ventricular fibrillation, when the hart quivers instead of pumping due to disorgarnized electrical activity in the ventricles. In the absence of treatment this followed by death. Interest has recently focused on the suggestion that autophagy may play a role under altered physiological conditions in various cardiovascular diseases including heart failure and ischemia/reperfusion. Reperfusion of the ischemic myocardium improves left ventricular dysfunction and survival, therefore, efforts must be made to improve recovery of myocardial function and minimize the risk of sudden cardiac death caused by ventricular fibrillation.

Autophagy is an evolutionary conserved process involved in degradation of long-lived proteins and excess or dysfunctional organelles. This pathway is constructed of four different follow up sections; (i) initiation, (ii) engulfment of damaged structures by a double-membrane structure, which is the autophagosome generation, (iii) the formation of autolysosome via the fusion of autophagosome and lysosome, and (iv) the final process is the degradation and recycling of the engulfed structures. Autophagy occurs constitutively at low levels under normal conditions in most cells, including cardiac myocytes and is an important house-keeping process in the cell. The role of autophagy in the cardiac tissue is apparently dual from the view of survival or death; it depends on

the type and the duration of stress. A consensus is now drawing the outcome of autophagic process “survival or death” depends on its intensity; a more intensified autophagy probably initiates a sequence of pathological reactions in myocardial cells and, ultimately, myocardial function derangement. Today, only few studies investigated a potential relationship between cardiac arrhythmias and modulation of autophagy. Some suggested that atrial fibrillation is associated with impaired cardiac autophagy, whereas others have shown that in severe mitral and tricuspid regurgitation, autophagy is increased in cardiomyocytes with or without atrial fibrillation. Although, evidence exists for a role of autophagy in the pathogenesis of ischemia-reperfusion-induced heart damages, some of them may be circumstantial, controversial or open to alternative interpretation.

Therefore, a major objective of our study was to investigate a possible connection between reperfusion-induced VF and autophagic processes furthermore, examination of electrically-induced VF on cardiac function, magnitude of infarct zone extent, and expression of proteins that are clinical and/or objective laboratory biomarkers for normal, versus disrupted heart function, necrosis, apoptosis, and autophagy. Thus, our investigation may offer further understanding of the arrhythmogenic mechanisms at molecular level and identify some trigger mechanisms responsible for arrhythmogenesis in the myocardium in the mirror of autophagic proteins. To answer these questions we did the following investigations:

- Investigate the autophagic activity during ischaemia-reperfusion caused ventricular fibrillation in isolated mouse heart model.
- Measurement the effect of electrically induced ventricular fibrillation to the autophagic process and heart functions in isolated working rat hearts model.
- Determine the expression of autophagic and apoptotic biomarkers during ventricular fibrillation by using Western blot and TUNEL method
- Investigate the effects of electrically induced ventricular fibrillation on infarcted size

II. Materials and methods

Animals

Male Sprague-Dawley rats weighing 370–400 g (Charles River International, Inc., Sulzfeld, Germany) were used for all experiments in the present study. The animals were housed at room temperature (approximately 25 ◦ C), with lighting set to alternating dark and light periods of 12 h. The rats were maintained on normal rodent chow and tap water *ad libitum*. Wild type male mice (25-35 g) were purchased from Charles River Laboratories International, Inc. (Sulzfeld, Germany). All animals received humane care, in compliance with the "Principles of Laboratory Animal Care" according to the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals, formulated by the National Academy of Sciences, and published by the National Institute of Health (NIH Publication No. 86-23, revised 1985). All protocols involving animal use were approved by the Committee of Animal Research, University of Debrecen, Hungary (3/2012/DEMAB, 21-03-2012).

Global myocardial ischaemia and reperfusion on isolated mouse hearts

In the first set of experiments, a global myocardial ischemia-reperfusion protocol was carried out. Hearts were mounted on a Langendorff isolated heart system and cannulated for perfusion with an oxygenated (95% O_2 / 5% CO_2) Krebs solution (37 °C) composed of (in mM) : NaCl 118.3, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, KH 2 PO 4 1.2, Glucose 11.1, $CaCl_2$ 2.5 (pH= 7.4). Hearts were perfused at a constant pressure (80 mmHg). Initially, hearts were allowed to stabilize for 30 min, and then 25 min global ischemia was initiated. Global ischemia was imposed by clamping the inflow line tube near to the aortic cannula. Subsequently, after global ischemia, the cardiac flow was reinitiated to allow reperfusion of the previously ischemic cardiac tissue for 80 min. ECG was registered by two silver electrodes attached directly to the heart. A computerized electrocardiogram (ECG) was obtained continuously during the protocol (ADInstruments, PowerLab, Castle Hill, Australia). The analysis of the ECG enabled the assessment of the incidence of VF. The heart was considered to be in irreversible VF if an irregular undulating baseline was present on the ECG at least for 3 min.

Isolated working rat heart preparation and electrical pacing protocol

Rats were anesthetized with intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg), and heparin (1000 U/kg) was then injected intravenously through the dorsal penile vein. After thoracotomy, hearts were excised, and placed into ice-cold perfusion buffer, with a composition described above. Perfusion of hearts was conducted using a 5 min washout period in Langendorff mode via the cannulated aorta, and then the left atrium was also cannulated. The perfusion fluid then passed to the left ventricle, from which it was spontaneously ejected through

the aortic cannula against a pressure equivalent to 100 cm of water. Hearts taken from the animals were segregated into test groups and subjected to 0 (Control), 1, 3, and 10 min of VF periods, respectively. Electrical fibrillation was carried out by 20 Hz (1200 beats/min) for 1, 3, and 10 min, respectively, using 5 V square-wave pulses of 2 ms duration. The active lead was attached to the apex of the heart and the ground lead was attached to the aortic cannula. These conditions suppressed the sinus node activity and resulted in VF consisting of an irregular undulating baseline on the ECG. Following the termination of electrical fibrillation, hearts were allowed post-fibrillation recovery period for 120 min. At the end of the 120 min of post-fibrillation recovery period, the expression or repression of the autophagic and apoptotic proteins were determined. Aortic flow, coronary flow, cardiac output, stroke volume, and heart rate were monitored. The aortic flow and coronary flow rate were measured by timed collection of the coronary perfusate that dripped directly from each heart. The cardiac output was determined as the sum of aortic flow and coronary flow in milliliters per min, from which stroke volume was additionally calculated.

Biochemical assays on mouse hearts

The expression level of LC3B, ATG-5, ATG-7, ATG-12, Bcl-2 and Beclin-1 proteins in left ventricular (LV) tissues was evaluated using Western immunoblotting. Proteins were isolated from heart tissue samples with homogenizing buffer (25 mM, Tris-HCl, pH 8, 25mM NaCl, 1mM Na-Orthovanadate, 10mM NaF, 10mM, Na-Pyrophosphate, 10nM Okadaic acid, 0.5mM EDTA, 1mM PMSF and 1x Protease inhibitor cocktail) with the help of a polytron homogenisator, centrifuged for 10 min at 4°C for 2000 rpm. The supernatant was transferred to a new tube and centrifuged for 20 min at 10000 rpm at 4°C and the supernatant was used as cytosolic fraction. Protein concentration was measured by a BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). Proteins were separated using electrophoresis on a polyacrylamide gel (12 or 15 % depending on the weight of the protein) and transferred onto a nitrocellulose membrane. Nonspecific binding sites were blocked with 5 % skimmed milk in Tris-buffered saline solution with 0.5 % Tween for 1 hour at room temperature. Membranes were then incubated overnight at 4 °C with primary antibodies directed against LC3B (1/333), ATG-7 (1/333), ATG-5 (1/1000), ATG-12 (1/1000), Beclin-1(1/1000), Bcl-2(1/1000), GAPDH (1/20000). After 3 washes, membranes were incubated with corresponding secondary antibodies for 1 hour at room temperature and signals were revealed by Western HRP substrate (Millipore, Billerica, MA, USA). Optical density of bands was quantified densitometrically after scanning and protein contents were expressed relative to GAPDH.

Western blot analysis on rat hearts

Protein expression levels for cleaved-caspase-3, p62, mTOR, phosphorylated -mTOR,LC3BII/LC3BI, and Atg5-12 complex proteins were evaluated using Western immunoblotting. Proteins were isolated from myocardial tissue with homogenizing buffer (25 mM Tris-HCl, pH 8.25 mmol NaCl, 1 mmol Na-Orthovanadate, 10 mmol NaF, 10 mmol Na-Pyrophosphate, 10 nmol Okadiac acid, 0.5 mmol EDTA, 1 mmol PMSF, 1 × protease inhibitor cocktail) using a polytron homogenisator on ice. Next, after 10 min centrifugation, 2000 rpm at 4 °C supernatants were transferred to fresh tubes and centrifuged for 20 min at 10000 rpm and 4 °C. The supernatants were used as cytosolic fractions. Protein concentration was measured by BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). Proteins were separated using 4–20% Mini-PROTEAN®TGX Stain-FreeTM Protein Gels then transferred onto polyvinylidene fluoride (PVDF) membranes. Non-specific binding was blocked using non-fat milk in Tris-buffered saline with 1% Tween20 for 30 min at room temperature. After washing with TBS-T the membranes were incubated overnight at 4 °C with primary antibodies against LC3B (1:1000), Atg5 (1:500), Atg12 (1:500), p62 (1:1000), cleaved-caspase3 (1:1000), mTOR (1:2000) and p-mTOR (1:2000). After 3 washes the membranes were incubated with secondary antibody for 1.5 h at room temperature and signal intensities for each protein band were detected using Clarity Western ECL Substrate (Bio-Rad, Hercules, CA, USA). The optical density of bands was measured using the ChemiDoc Touch Imaging System and protein levels determined with the Bio-Rad Image Lab 5.2 software (Bio-Rad). The levels of the investigated proteins were normalized against the total protein loaded on the gels. The protein expression was quantified by the ratio of (band volume)/(total protein volume). Thus, this method eliminates the need for housekeeping proteins.

Infarction zone magnitude in VF-treated isolated rat hearts

Triphenyl-tetrazolium chloride (TTC) staining was used to determine infarcted areas. For these experiments, hearts were subjected to 0 (Control), 1, 3, and 10 min of VF followed by 120 min of post-fibrillated perfusion. A total of 100 mL of 1% TTC solution in phosphate buffer (Na_2HPO 4,88.05 mmol, NaH_2PO 41.8 mmol) was heated to 37 °C and administered directly via the side arm of the aortic cannula. TTC-stained viable myocardium was deep red, while potentially infarcted areas remained pale, allowing clear demonstration and measurement of infarction magnitude. Hearts were stored at −80 °C for a subsequent analysis and then sliced transversely to the apico-basal axis into 2–3 mm thick sections, weighted, blotted dry, and placed in between microscope slides, then scanned at 600 dpi on a Hewlett-Packard Scanjet single pass flatbed scanner (Hewlett-Packard, Palo Alto, CA, USA). Using ImageJ software, each image was subjected to equivalent degrees of background subtraction, brightness, and contrast enhancement for improved

clarity and distinctness. Infarct area (pale areas, white pixels) of each slice were traced and the respective areas were calculated by pixel density analysis. Infarcted areas and total area were measured by computerized planimetry software. Infarct size was expressed as a percentage ratio of the infarcted zone to the total area in each heart (percentage of pixels).

Fluorescens cell death detection

To detect apoptosis, we used the terminal deoxynucleotidyl transferase (TdT) nick end labelling test by the In Situ Cell Death Detection Kit, TMR (fluorescein-labeled cell markers) red (Roche, Mannheim, Germany). Apoptosis can be detected by labeling the free 3 0 -OH termini with modified nucleotides in an enzymatic reaction. The enzyme terminal deoxynucleotidyl transferase (TdT) catalyzes the template-independent polymerization of deoxyribonucleotides to the 3 0 -end of single- and double-stranded DNA. Harvested hearts tissue were fixed in 4% formalin for 24 h at 4 °C, embedded in paraffin, and cut into 4.5 μm thick sections. All tissue sections were deparaffined in xylene and acetone then rehydrated in 70% ethanol and water. The sections were boiled in citrate buffer pH 6.0 for 12 min, then cooled at room temperature for 20 min, thereafter, washed two times for 5 min in phosphate-buffered saline (PBS pH 7.4). Finally, sections were incubated with TdT (terminal deoxynucleotidyl transferase) in a humidified box, at 37 °C for 1 h. After washing, to identify nuclei, we used DAPI (4 0 ,6-diamidino-2-phenylindole), which emits blue fluorescence upon binding to AT regions of DNA (Thermo Fisher Scientific, Waltham, MA, USA). The slides were then washed with PBS, after being air-dried, and subsequently covered with mounting medium and glass slide covers. Moviol solution was used as a mounting medium. Fluorescence microscopic images were obtained by a Zeiss AxioScope A1 microscope, EC Plan-Neo fluar 40x /0.75 M27 objective lens with HBO100 illuminator and Zeiss AxioCam ICm1 camera (Zeiss, Jena, Germany). After merging the blue and red channels, purple spots were associated with apoptotic nuclei, while blue spots were identified as non-apoptotic nuclei (ZEN 2012 software, Zeiss). Apoptosis was quantified by ratio of TdT-positive nuclei/total nuclei in each section.

Statistic

Numerical data corresponding to magnitude of each outcome evaluated were expressed as means or mean fractions of baseline ± Standard Error (S.E.). Results were analyzed using adapted Student T tests and repeated measures ANOVA if necessary. If significant changes were found, a Tukey-Kramer test was applied. A level of $p<0.05$ was considered statistically significant. Difference of means was onsidered significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Statistical analysis was carried out with GraphPad Prism 6.07. (GraphPad Software Inc., San Diego, CA, USA).

III. Results

ECG measurements and incidence of VF in isolated, ischemic-reperfused mouse hearts

The outcome of 25 minutes global ischemia followed by 80 minutes of reperfusion (I/R) applied to isolated working hearts from 21 mice. 8 hearts exhibited VF in response to I/R treatment, 13 remained VF-free. Coronary flow (CF) rate measured in the non-VF group was 3.3 ± 0.4 ml.min -1 versus 3.1 ± 0.6 ml.min -1 in hearts exhibiting VF. Although time course measurements of post-I/R CF suggested a trend toward exacerbated reduction of this parameter at the 100 minutes time point, the overall difference in CF for the time course evaluated was non-significant.

Western blot analyses for autophagy- and apoptosis associated gene products in mouse hearts

Investigating the autophagy processes, LC3B-I is converted to LC3B-II through lipidation by a ubiquity-like system involving Atg7 and Atg3 that allows for LC3B to become associated with autophagic vesicles. The presence of LC3B in autophagosomes and the conversion of LC3B-I to the lower migrating form of LC3B-II were used as indicators of autophagic processes. Although no significant difference in LC3B-I expression was noted in VF versus non-VF (Control) tissue, LC3B-II expression in tissue from VF-afflicted hearts was observed to be significantly greater than in non-VF control hearts $p<0.05$). Moreover, elevated LC3B-II without change in LC3B-I content resulted in LC3B-II/LC3B-1 ratios in tissues from VF hearts that were significantly elevated relative to these ratios observed in non-VF control hearts ($p<0.05$). No significant correlation of ventricular fibrillation and Atg expression was observed in our *in vitro* model. Indeed, no significant difference was detected in the cellular expression of Atg-7 and Atg-5. In addition, the expression of Atg5-Atg12 conjugation was also unchanged. Increased activation of autophagy may be due to the alteration of Beclin-1 expression. Indeed, Beclin-1 is a key protein in the regulation of autophagy which appears to be increased following myocardial fibrillation. It is well known that Beclin-1 activation is mainly regulated by the inhibition of Bcl-2 protein. However, no change was detected in its expression in our model.

Western blot analyses of autophagic- and apoptotic related proteins in rat hearts

The level of p62 in hearts subjected to 1, 3, and 10 min VF stimulation periods followed by 120 min perfusion and expressed by approximately 1/3 (** $p < 0.01$), 1/2 (*** $p < 0.001$), and 2/3 (** $p < 0.001$) less of this protein, respectively. Expression levels of P-mTOR/mTOR ratio were determined as a function of VF stimulation periods and its ratio showed a gradual increase with the extended duration of electrical VF. It is also shown that relative to the unstimulated control group, no significant difference for the average ratio of P-mTOR/mTOR ratio was observed in hearts subjected to 1 min of electrical VF. This value was approximately 1/4 higher in hearts subjected to 3 min of VF period (* $p < 0.05$) and further increased in hearts subjected to 10 min of VF (* p <

0.05). The time-dependent effect of VF on the expression of LC3BII/LC3BI protein ratio by tissue from isolated perfused hearts results revealed that relative to the unstimulated Control group, hearts subjected to 1, 3, and 10 min of VF periods showed no significant changes in this protein expression, although a gradual decrease was registered. The average expression levels of Atg5-12 relative to the control group receiving no VF stimulation no significant differences in average expression of this protein complex were observed for hearts subjected to 1 min and 10 min periods of VF. However, the average Atg5-12 expression levels in hearts subjected to 3 min of stimulated VF were approximately increased by 25% ($p<0.05$) in comparison with the non-fibrillated control group.

Effects of pacing-induced VF on infarct size, caspase-3 activation and TUNEL positivity

The duration of electrical stimuli induced ventricular fibrillation could be contributed to the depression of cardiac function including aortic flow, coronary flow, and stroke volume changes. Decreased cardiac function was accompanied by enlarged infarcted volume. Compared to the Control hearts subjected to no VF stimuli, the approximately 10-fold greater infarcted zone extent was detected in hearts subjected to 10 min duration of VF, which may be accounted for the capacity of electrically-induced VF to promote pathway-mediated oxidative damage in heart tissue as described earlier. The longest duration of electrically-induced VF was detrimental to the development of infarcted myocardium as TTC staining discriminates between the living and dead tissues. The known mechanisms by which infarcted zone expansion is triggered and augmented, strongly suggests degradation of metabolite compartmentalization within cardiomyocytes and other cardiovascular cells, resulting in both arrhythmogenesis and derangement of cardiac function. An enhanced number of TUNEL positive cells were observed in all electrically fibrillated hearts. The most intense TUNEL positivity was observed in hearts undertaken to 3 min of VF followed by 120 min of post-fibrillated perfusion. These observations were in line with the cleaved caspase-3 results indicating the highest cleaved caspase-3 expression in hearts undertaken to 3 min of ventricular fibrillation. Caspase-3 activation is an early event in the apoptotic cell death cascade, and very high activity of this enzyme may trigger disease through excessive apoptosis in the intact myocardium.

IV. Conclusion

Different types of arrhythmias arise as a consequence of myocardial ischemia, reperfusion, and genetic mutations. Interest in the complications of myocardial ischemia has resulted in much emphasis being placed upon the characterization and treatment of supraventricular and ventricular arrhythmias. However, the realization that the mechanisms of reperfusion-induced arrhythmias are quite distinct from those associated with the mutation in cardiac ion channel genes, and the growing awareness of genetically derived arrhythmias as a clinical encountered event, has done much to readdress the balance of research interest. Myocardial ischemia followed by reperfusion elicits different changes in the expression or repression of several genes, opening and closing cardiac ion channels, myocardial function, and arrhythmogenesis in the hear. Although, the electrophysiology and arrhythmogenesis of the myocardium including the mechanisms of ventricular arrhythmias are relatively very well defined, currently only few data are available to establish a connection between cardiac arrhythmias and autophagic responses. The role of autophagy in arrhythmogenesis can be appreciated by considering a common feature of heart arrhythmias associated with myocardial ischemia, reperfusion, and genetic mutations; specifically, presence of toxic protein aggregates in cells of affected tissue.

In mouse model we have observed an elevated level of Beclin-1 in the fibrillated myocardium in comparison with the nonfibrillated hearts, suggesting an overwhelming activation of autophagy during ventricular fibrillation. Based on our data we assure that during fibrillation a stronger upregulation of Beclin-1 indicates a more intensified autophagy. Moreover, Beclin-1 along with caspases including caspase- 3, -7,- 8 is playing a role in the crosstalk between apoptosis and autophagy. Another molecule that regulates the balance between tissue levels of apoptosis and autophagy is the Bcl-2, which acts as a negative regulator of apoptosis that additionally interacts with Beclin-1 to inhibit autophagy with resulting promotion of inflammasome activity. The level of Bcl-2 might not substantially alter in response to acute tissue damage such as I/R injury. Such an outcome is consistent with the outcome of experiments, demonstrating no significant difference in Bcl-2 expression within I/R-injured tissue experiencing VF, versus myocardium from hearts that remained VF-free. The data obtained from the comparison of ratio of LC3 B-I/LC3B-II, and comparing the level of LC3B-II content of the groups support the idea regarding the more intensified autophagy in the fibrillated myocardium. Interestingly no significant correlation was observed between occurrence of VF in I/R-injured heart tissue and expression of autophagy-related gene (Atg) products Atg7, Atg5 and Atg12-Atg5 in I/R-injured myocardial tissue. These proteins participate in autophagy through a ubiquitin-like conjugation system in which Atg12 is covalently bound to Atg5 and targeted to autophagosome vesicles. The results demonstrate that heart tissue

responding to I/R injury with VF experiences significantly greater levels of autophagy than I/R injured hearts that remained free of arrhythmias. However the major questions e.g., autophagy contributes to the development of VF; or VF induces autophagy; or in vulnerable hearts VF develops regardless autophagy, and these hearts due to vulnerability are not able to maintain the autophagy in the appropriate level are remained unanswered.

The further investigations have revealed several features of heart tissue response to electrically-stimulated VF that may prove enormously useful in ongoing exploration of arrythmogenesis, with particularly valuable insight into autophagy-related pathophysiology. A major positive outcome, as this has improved understanding of both the usefulness and limitations of the electrically-stimulated VF model of the isolated working rodent heart, in which the authors have relied on extensively as an investigative tool over several decades of cardiovascular research. It is here acknowledged that although some of its aspects may depart from being precisely analogous to *in vivo* conditions, the tissue environment and resulting arrhythmogenic consequences provided a close enough parallel to the failing vertebrate heart as to allow clinically relevant conclusions to be drawn. Further, results of this report which demonstrated variable post-VF capacity for recovery of normal rhythm from heart to heart, underscored the caution that cardiologists may need to exercise in tailoring therapy to individual patients. Observations of VF effects on cardiac infarction expanded the scope of potential use for inducers of endogenous defenses including apoptosis and autophagy as a countermeasure to this effect; and demonstration of VF effects on biomarkers for apoptosis, and autophagy-associated proteins open novel directions in approaches to intervention in arrhythmogenic influences by which each process may be manipulated to address a wide range of disease states, extending on cardiovascular medicine.

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/279/2019.PL
Subject: PhD Publikációs Lista

Candidate: András Czeglédi
Neptun ID: Z08Z2A
Doctoral School: Doctoral School of Pharmacy

List of publications related to the dissertation

1. **Czeglédi, A.**, Tósaki, Á., Gyöngyösi, A., Zilinyi, R., Tósaki, Á., Lekli, I.: Electrically-Induced Ventricular Fibrillation Alters Cardiovascular Function and Expression of Apoptotic and Autophagic Proteins in Rat Hearts.
   Int. J. Mol. Sci. 20 (7), 1628-, 2019.
   DOI: http://dx.doi.org/10.3390/ijms20071628
   IF: 4.183 (2018)

2. Meyer, G., Czompa, A., Reboul, C., Csépányi, E., **Czeglédi, A.**, Bak, I., Balla, G., Balla, J., Tósaki, Á., Lekli, I.: The Cellular Autophagy Markers Beclin-1 and LC3B-II are Increased during Reperfusion in Fibrillated Mouse Hearts.
   Curr. Pharm. Des. 19 (39), 6912-6918, 2013.
   IF: 3.288

DEBRECENI EGYETEM
Egyetemi és Nemzeti Könyvtár

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

List of other publications

3. Nagy, F., Tóth, Z., Bozó, A., **Czeglédi, A.**, Rebenku, I., Majoros, L., Kovács, R. L.: Fluconazole is not inferior than caspofungin, micafungin or amphotericin B in the presence of 50% human serum against Candida albicans and Candida parapsilosis biofilms.
   Med. Mycol. 57 (5), 573-581, 2019.
   DOI: http://dx.doi.org/10.1093/mmy/myy108
   IF: 2.851 (2018)

4. Gyöngyösi, A., Zilinyi, R., **Czeglédi, A.**, Tósaki, Á., Tósaki, Á., Lekli, I.: The role of autophagy and death pathways in dose-dependent isoproterenol-induced cardiotoxicity.
   Curr. Pharm. Design. [Epub ahead of print], 2019.
   DOI: http://dx.doi.org/10.2174/1381612825666190619145025
   IF: 2.412 (2018)

5. Zilinyi, R., Czompa, A., **Czeglédi, A.**, Gajtkó, A., Pituk, D., Lekli, I., Tósaki, Á.: The Cardioprotective Effect of Metformin in Doxorubicin-Induced Cardiotoxicity: the Role of Autophagy.
   Molecules. 23 (5), 1-12, 2018.
   DOI: http://dx.doi.org/10.3390/molecules23051184
   IF: 3.06

6. Czompa, A., Gyöngyösi, A., **Czeglédi, A.**, Csépányi, E., Bak, I., Haines, D. D., Tósaki, Á., Lekli, I.: Cardioprotection afforded by sour cherry seed kernel: the role of heme oxygenase-1.
   J. Cardiovasc. Pharmacol. 64 (5), 412-419, 2014.
   DOI: http://dx.doi.org/10.1097/FJC.0000000000000132
   IF: 2.135

Total IF of journals (all publications): 17,929
Total IF of journals (publications related to the dissertation): 7,471

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

05 July, 2019